DECREASED UDP-GLUCURONIC ACID IN RAT LIVER AFTER ETHER NARCOSIS

An isotachophoretic study

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1. Introduction

For the past several years our laboratory has studied the effect of protein deficiency and cytostatics on the RNA metabolism of the liver in rat. With capillary isotachophoresis a fast screening method for determination of acid-extracted nucleotides was developed [1]. In that work unanaesthetized rats were killed by the double hatchet method [2] and the liver fragments obtained were rapidly frozen. This method seems to be the most accurate but we did not succeed in keeping the time from hatchet stroke to freeze-clamping [3] sufficiently short and constant. A sampling time of >7 s gave low nucleotide triphosphate concentrations and the ATP/ADP ratio dropped below 2.0.

Therefore, other methods of liver sampling, namely under diethylether or divinylether narcosis, were also tried primarily to obtain better reproducibility with high and constant ATP/ADP/AMP ratios. Unexpectedly these anaesthetic agents lowered the UDP-GlcUA level of the liver, a finding which prompted this publication.

2. Materials and methods

2.1. *Chemicals*

All nucleotides and substances for determining the UV peaks, β-alanine and *n*-caproic acid were purchased from Sigma Chemical Co. USA. Other materials were obtained as follows: HPMC, Dow Chemicals, USA; diethylether (Aether ad Narcosin), Skånska Bomullskrutfabriken AB, Sweden and divinylether (Vinydan), H. Lundbeck, Denmark.

Abbreviations: UDP-GlcUA, UDP-glucuronic acid; HPMC, hydroxypropylmethylcellulose

2.2. *Animals and liver sampling*

Fourty-six male Wistar rats (~150 g) were fed on a 25% casein diet [4] for 7 days. At about 8 a.m., 22 animals were anaesthetized in their cages placed inside a ventilated work bench. A funnel was put over the rat and oxygen was bubbled through the anaesthetic agent at 2 l/min. Diethylether (~4 ml) or 1.5 ml divinylether were necessary to achieve surgical anaesthesia. As soon as the rat was anaesthetized, it was transferred to a cork plate. In experiments where the rat was operated immediately, the anaesthetic administration was continued through a few layers of loosely fluffed gauze. The shortest time from the beginning of anaesthesia with diethylether and divinylether to sampling of liver tissue was 90 s and 60 s, respectively. For longer periods of anaesthesia the method with a plastic bag was used [5]. The abdomen was quickly opened, the left lobe of the liver was cut out with a pair of scissors and rapidly freeze-clamped between two metal blocks, precooled in liquid N_2. The time from cutting to freezing was <1 s.

Liver sampling under narcosis was compared to the 8 rats of 24 killed with the double hatchet method [2] with the highest ATP/ADP ration (table 1). The sampling time for the 8 rats was 3–7 s [1].

2.3. *Extraction and determination*

The extraction with cold perchloric acid–methanol and neutralization with KOH were as in [1]. The quantitation was made with isotachophoresis on an LKB 2127 Tachophor (LKB, Sweden) equipped with a UV lamp at 254 nm. The separation was run in an 81 cm tube at 20°C. The electrolytes were 5 mM HCl + 0.25% HPMC + β-alanine to pH 3.89 and 10 mM caproic acid. Spacer solution [1] was used increase separation. Additional to 5 μl extract, 1.5 μl following

spacer solution was added: 0.4 mM tartaric acid; 0.5 mM fumaric acid; 0.4 mM chloroacetic acid; 0.3 mM trichloroacetic acid; 0.2 mM glucaric acid; 0.3 mM glycolic acid; 0.3 mM glyceric acid; 0.5 mM gluconic acid; 0.3 mM acetic acid; 0.3 mM laevulinic acid; 0.8 mM propionic acid. The peaks were monitored with an LDC 304-50 computing integrator (Laboratory Data Control, England).

2.4. *Calibration*

UTP, GTP, ATP and CTP were calibrated simultaneously in concentrations normally occurring in rat liver. Phosphoenol pyruvic acid (0.25 nmol) was added as spacer between UDP and GTP, and trichloroacetic acid (0.50 nmol) between ATP and CTP. All calibration curves had correlation coefficients of 0.9985 or better. UDP-GlcUA was also calibrated with a correlation coefficient of 0.9999.

3. Results and discussion

Fig.1 shows two scans from liver extracts, sampled with the double hatchet method and under diethylether narcosis. The ATP/ADP/AMP ratios are equal and high (15/4/1) indicating good methodologic conditions. The striking difference between the two scans is the heavy decrease of UDP-GlcUA for the sample collected under diethylether narcosis. Fig.2 shows the relation between length of anaesthesia and amount of UDP-GlcUA. Diethylether decreased the UDP-GlcUA in <90 s and the pool was 20% of normal after 5 min anaesthesia. Divinylether, which gives quicker surgical anaesthesia, was also tested. The depression was not so pronounced, and 60 s anaesthesia gave almost normal values of UDP-GlcUA and UTP.

Workers using diethylether narcosis for liver sampling find low concentrations of UDP-GlcUA [6,7] compared to our results (table 1). With cervical dislocation values in the same range as ours are obtained [8,9].

One explanation for the decrease of the UDP-GlcUA pool might be that the products from the metabolism of ether [10,11] affect the pathway. Microsomal *O*-dealkylation [12] gives ethanol which can participate in glucuronidation [13,14] or change the NADH/NAD and NADPH/NADP ratios resulting in decreased UDP-

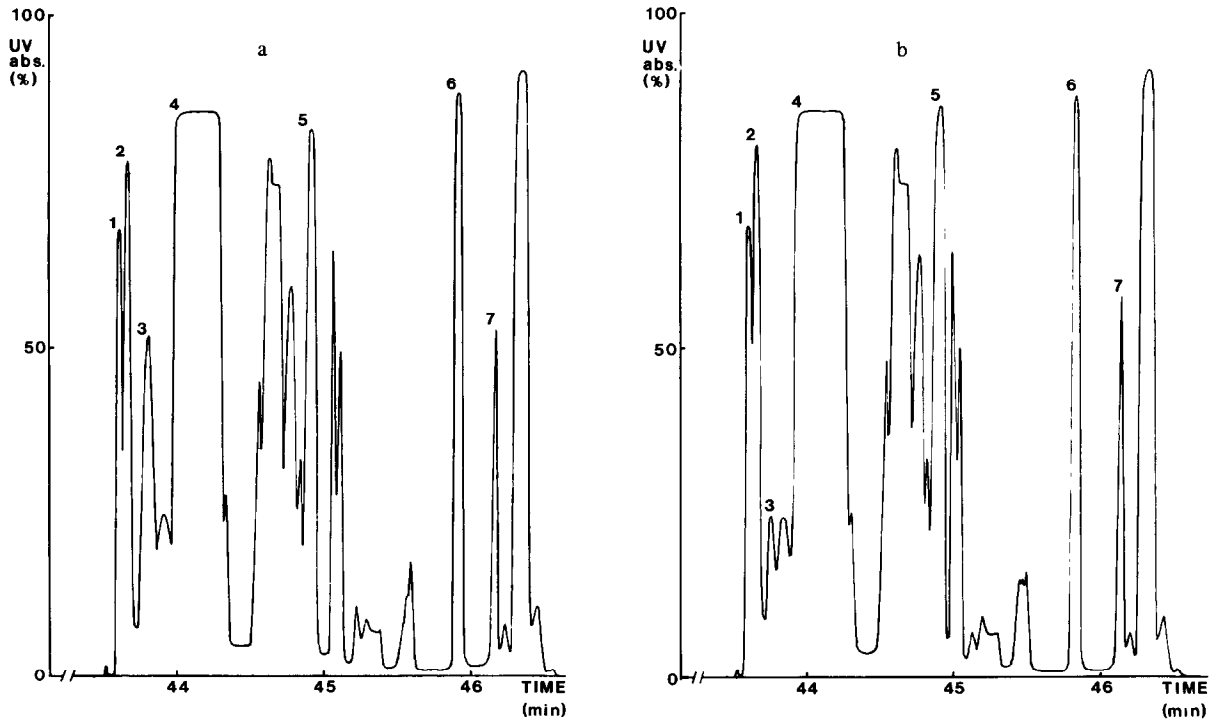

Fig.1. Isotachophoretic records from liver extracts sampled (a) with the double hatchet method and (b) under diethylether anaesthesia. Extract (5 μl) with 1.5 μl spacer solution was run in an isotachophoretic system and detected at 254 nm. (1) UTP; (2) GTP; (3) UDP-GlcUA; (4) ATP; (5) ADP; (6) ascorbic acid; (7) AMP. Other peaks in the records were described in [1].

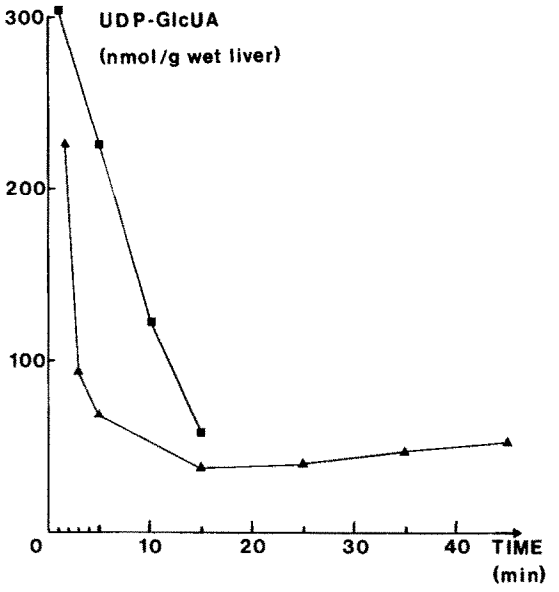

Fig.2. Decreased UDP-GlcUA (nmol/g wet liver) at extended anaesthesia with diethylether (▲) and divinylether (■). The low therapeutic index of divinylether made longer periods of anaesthesia difficult.

GlcUA formation and in increased flow of glucuronic acid to ascorbic acid [15].

A more likely explanation is a non-covalent regulatory mechanism [16], by which ether may affect the microsomal membrane resulting in an increase of active enzyme sites. In [17] phospholipase A treatment of microsomes resulted in a 6-fold increase in the UDP-GlcUA transferase activity. Triton X-100 and other agents are also capable of influencing the protein–lipid interaction [18].

An induction of enzyme synthesis caused by exposure to diethylether [19] cannot explain our findings as the induction interval is too short. The production of free glucuronic acid via the pyrophosphatase–phosphatase route is another explanation but no glucuronic acid 1-phosphate was detected in the isotachogram.

In dogs anaesthetized for 2 h with diethylether, there was a 15-fold increase of ascorbic acid in liver urine over 24 h [20,21]. The following day the values were normal. The results from rats were somewhat lower. This agrees with our observation that the ascorbic acid pool in liver is elevated for animals anaesthetized 15–45 min compared to animals anaesthetized 90 s or animals in the double hatchet group ($0.001 < p < 0.01$).

In table 1 ribonucleotide concentrations are shown. The double hatchet method gave lower amounts of triphosphates probably because a sufficiently short time between killing and freezing (2.5–3.5 s) was difficult to achieve. In addition, there was no definite relation between sampling time and the ATP/ADP ratio. Factors as stress before killing and how and

Table 1
Nucleotide concentrations with different methods of liver sampling

	Double hatchet	Divinylether	Diethylether
Sampling time (s)	3 – 7	< 1	< 1
UTP	378 ± 7	379 ± 7	459 ± 12[a]
GTP	421 ± 9[a]	499 ± 5	464 ± 9[b]
UDP-GlcUA	332 ± 20	322 ± 15	235 ± 13[b]
ATP	2693 ± 46[a]	3234 ± 46	3186 ± 23
ADP	1161 ± 47[a]	919 ± 25	841 ± 29
AMP	348 ± 23[a]	197 ± 16	258 ± 21
Σ (AMP, ADP, ATP)	4202 ± 66	4350 ± 54	4285 ± 39
Energy charge	0.779 ± 0.008[a]	0.849 ± 0.005	0.842 ± 0.006
No. animals	8	6	6

[a] Significance compared to the divinylether group; $p < 0.001$
[b] Significance $0.001 < p < 0.01$

The shortest possible time of anaesthesia was used, that is 60–70 s and 90–100 s for the two agents, respectively. Values are given as means ± SEM (nmol/g wet liver tissue).

$$\text{Energy charge} = 0.5 \times \frac{2\,\text{ATP} + \text{ADP}}{(\text{ATP} + \text{ADP} + \text{AMP})} \qquad [23]$$

where the hatchet stroke divided the liver may interfere. The advantages of anaesthesia were the constant and short sampling time (<1 s) and that the same liver lobe, the left lateral, was taken. This method gave higher amounts of nucleotide triphosphates and better reproducibility. Divinylether is an alternative for short operations. Its advantages over diethylether are a shorter anaesthesia induction time, a lesser influence on UDP-GlcUA and, consequently, less influence on UTP. Additionally, divinylether is less irritating for the respiratory tract and less excitatory.

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